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(71) Applicant: TKB ASSOCIATES LIMITED PARTNERSHIP [US/US]; Suite 102, 3550 Lander Road, Pepper Pike, OH 44124 (US).

(72) Inventors: KAPLAN, David, R.; 2854 Sedgewick Road, Shaker Heights, OH 44120 (US). TYKOCINSKI, Mark, L.; 22225 Douglas Road, Shaker Heights, OH 44122 (US).

(74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon, First Interstate World Center, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US).

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(54) Title: METHODS FOR ENGINEERING ARTIFICIAL VETO CELLS

(57) Abstract

A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigen or processed antigen comprising the steps of providing an antigen presenting cell having the alloantigen or processed antigen, and externally contacting the extracellular surface of the antigen presenting cell with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind the chimera to the surface of the antigen presenting cell in a manner which presents the polypeptide on the cell's surface such that the polypeptide is able to reduce T-cell proliferation, cytotoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

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# DESCRIPTION

## METHODS FOR ENGINEERING ARTIFICIAL VETO CELLS

### Field of the Invention

This invention relates to methods and reagents for engineering artificial veto cells ("AVCs") for purposes of immunotherapy.

### Background of the Invention

The following is a general discussion of relevant art, none of which is admitted to be prior art to the invention.

Antigen-specific (hereinafter referred to as "specific") immunotolerization is a therapeutic endpoint in subjects in need of the selective suppression of untoward immune responses to defined antigens. Given the centrality of pathogenic T lymphocytes in autoimmune, alloimmune, and other acute and chronic inflammatory diseases, an objective in the field of immunotherapy is the development of strategies for inhibiting specific T-cells. Antigen-presenting cells ("APCs") such as dendritic cells, B lymphocytes and macrophages provide one efficient means for accessing antigen-specific T-cells (T lymphocytes).

In general, APCs are responsible for initiating most immune responses through their pivotal role in antigen presentation to T-cells. During antigen presentation, endogenously processed nominal antigen peptides associate intracellularly with either class I major histocompatibility complex ("MHC") heterodimers or with class II MHC heterodimers, and the resulting heterotrimeric complexes are then translocated to APC surfaces (reviewed in Germain and Margulies, 11 Annu. Rev. Immunol. 403, 1993; Germain, 76 Cell 287, 1994). For a single immuno-

genic protein that is pulsed with APCs, processing frequently yields a diverse MHC:nominal antigen peptide repertoire, the composition of which may differ between different APCs (Falk et al., 348 Nature 248, 1990; Falk et al., 174 J. Exp. Med. 425, 1991; Henderson et al., 255 Science 1264, 1992; Henderson et al., 90 Proc. Natl. Acad. Sci. USA 10275, 1993; Hunt et al., 255 Science 1261, 1992; Rammensee et al., 11 Annu. Rev. Immunol. 213, 1993; Huczko et al., 151 J. Immunol. 2572, 1993).

In order to achieve a more defined MHC:nominal antigen peptide repertoire on a given APC surface, it is experimentally feasible to load exogenously synthesized oligopeptides onto class I or class II MHC heterodimers (Harding et al., 86 Proc. Natl. Acad. Sci. USA 4230, 1989). Such exogenously-loaded peptides do not require intracellular proteolytic processing.

For purposes of antigen presentation, it is common to employ one of two categories of cells, that is, cells that naturally express MHC heterodimers and cells transfected with an MHC gene expression cassette. In principle, another category of an MHC-bearing cell is one in which the MHC molecule has been exogenously attached to the cell surface. A method for delivering an MHC molecule to an APC surface has been described which entails the use of an HLA-A2.1-streptavidin chemical conjugate which can be added to a pre-biotinylated cell (Elliott and Eisen, 87 Proc. Natl. Acad. Sci. USA 5213, 1990). The chemical moiety was shown to be alloantigenic, but no data was presented that it can bind and present a nominal antigen peptide.

Functional interactions between APCs and T-cells are known to be mediated by both cell surface-associated and soluble molecules. Antigen-specificity in this interaction is provided by the major histocompatibility complex:nominal antigen peptide complex of the APC interacting with the T-cell receptor (TCR) of the T-cell,

constituting a trimolecular axis. However, effective interactions additionally require certain cell surface-associated and soluble costimulator molecules of APCs binding to receptors for these costimulators on T-cells. Examples of known costimulators on APCs are B7 (Linsley et al., 87 Proc. Natl. Acad. Sci. U.S.A. 5031, 1990); ICAM-I (van Seventer et al., 144 J. Immunol. 4579, 1990); VCAM-I (van Seventer et al., 174 J. Exp. Med. 901, 1991); LFA-3 (van Seventer et al., 21 Eur. J. Immunol. 1711, 1991; fibronectin (Shimizu et al., 145 J. Immunol. 59, 1990; Nojima et al., 172 J. Exp. Med. 1185, 1990; Davis et al., 145 J. Immunol. 785, 1990).

Tykocinski and Kaplan, U.S. Patent No. 5,242,687 disclose a method for effecting conversion of a T-cell activator into a T-cell inhibitor by expressing a CD8 polypeptide on the surface of an APC. In this case, the CD8 molecule functions as a "coinhibitor", triggering an inhibition program within the T-cell.

## Summary of the Invention

The present invention provides different methods and compositions for molecularly engineering APCs to tailor their T-cell modulatory properties, for both in vivo and ex vivo applications. Applicant has determined a broad set of methods for converting APCs from T-cell activators into T-cell inhibitors. An APC functioning in an T-cell inhibitory mode can be referred to as either a "deletional APC" (since it is deleting a specific T-cell through either the induction of anergy or apoptosis) or a "veto cell" (since it is vetoing a specific T-cell in a manner that mimics the natural veto function of an ill-defined mononuclear cell population in the periphery). Previously, CD8 coating was the only known method for engineering AVCs. The present invention offers a series of additional methods for generating non-naturally occurring veto cells, establishing the concept that "artificial

veto cells (AVCs)" constitute a broader class of cells that can be purposefully engineered through diverse methods.

A key aspect of the present invention is that the APC is being used as a delivery vehicle for an inhibitory reagent. The APC provides an efficient means for specific T-cell targeting, thereby focusing the reagent upon specific T-cell responders that are engaged by the antigenic peptides presented at the APC surface. Hence, the antigen presentation function of the APC is being used to advantage. By this approach, the inhibitory molecular reagent can be delivered locally, bypassing the need to administer it systemically and thus avoiding potential toxicities associated with systemic administration. Thus a critical aspect of the present invention is that the T-cell modulator is acting locally, either through cell-to-cell contact or local transit.

Another advantage is that the inherent capacity of the APC to process and present diverse antigenic peptides is being exploited. This obviates the need for prior knowledge of the precise antigenic peptides within a complex protein mixture, such as a cell extract, that are responsible for T-cell pathogenesis. AVCs can thus be pulsed with extracts from target tissues of a given disease, and the AVC (via its APC function) can present the diverse repertoire of potential pathogenic peptides to the T-cell population of the patient.

Applicant has determined those molecules that can be newly expressed on or in APCs in order to convert them into AVCs. Reagents useful for AVC engineering include both cell surface-associated and soluble molecules. Applicant believes that in each instance an inhibitory reagent provided by the APC binds to a receptor for the reagent on the T-cell, and this binding event serves to trigger an inhibitory program within the T-cell.

Applicant herein discloses that any one of a

number of molecules with known T-cell inhibitory activity can be used as a reagent for AVC engineering. Agents known to induce apoptosis in T-cells or other types of cells constitute candidates. A preferred polypeptide for AVC generation is Fas ligand ("Fas-L") (also known as APO-1 ligand) which is known to be capable of inducing apoptosis in both resting and activated T-cells via signaling through Fas (the Fas-L receptor, Fas antigen) molecules on the T-cell surfaces.

Other T-cell apoptotic pathways can be utilized as well. For example, it is known that soluble antibodies with specificity for either the class I MHC heavy chain or the class I MHC $\beta_2$ microglobulin light chain can inhibit the proliferation and triggering of cytotoxicity of T-cells, and it is further believed that in at least some contexts such antibodies evoke T-cell apoptosis. Hence, according to the present invention, another preferred reagent is an anti-class I MHC antibody or an anti-$\beta_2$ microglobulin antibody expressed by or on the AVC. Alternatively, to avoid having to use heterodimeric antibody molecules and to thereby simplify the application of the approach with a smaller reagent, one can generate single chain anti-class I Fvs that incorporate the heavy and light chain variable regions obtained from the corresponding polypeptides into a single polypeptide chain. There are numerous examples for the successful production of soluble functional Fvs directed against other target molecules, and these provide clearcut guidelines for the design and production of functional Fvs. Both cell surface-associated and soluble forms of the antibodies or Fvs can be employed.

In addition to known apoptosis inducers, other molecules with T-cell inhibitory potential can be employed for AVC engineering. Such molecules may either act directly on the T-cell or may modulate other molecular systems of the AVC itself, conferring or enhancing the T-

cell inhibitory action of the AVC. Preferred reagents in this category include known immunosuppressive cytokines, for example, interleukin-10 ("IL-10"), transforming growth factor $\beta$, heterodimeric placental protein 14, homodimeric/monomeric placental protein 14, and viral proteins. Examples of an immunoregulatory viral gene products include those originating from the human immunodeficiency virus (HIV). One example of a HIV immunoregulatory protein is tat, but HIV immunoregulatory proteins are not limited to tat. These various proteins share in common T-cell inhibitory activity, though they differ in their respective inhibitory mechanisms.

Applicant discloses that AVC engineering can be accomplished through both gene and protein transfer. Gene transfer is especially well-suited for expressing immunosuppressive genes in AVCs that encode for the production of secreted proteins, but is obviously also applicable to the expression of cell surface reagents. There is broad literature to guide one in configuring appropriate expression systems for gene transfer into AVCs. This includes integrating vectors (for example, retroviral, adenoviral, adeno-associated viral, and naked DNA vectors), episomal (extrachromasomally-replicating) vectors (for example, Epstein-Barr virus and BK virus vectors), and cytoplasmic expression vectors (for example, T7 promoter/polymerase vectors). Though ex vivo gene transfer, followed by administration of the engineered AVCs to a patient, is a preferred therapeutic application of the present technology, one can readily envision in vivo gene transfer applications with cell-specific targeting vectors.

AVC engineering can also be accomplished by protein transfer, that is, the external application of a cell surface-associating or soluble protein. In the case of cell surface-associating proteins, artificially lipid-modified variants of polypeptides are preferred. Lipid

modification can be accomplished by covalently conjugating lipids to soluble derivatives of the inhibitory protein of interest. An example of a lipid modification is palmitoylation. An advantage of this approach is that soluble recombinant proteins can be readily produced in large quantities using yeast or bacterial expression systems.

Alternatively, artificial glycosyl-phosphatidylinositol ("GPI")-modified derivatives of proteins can be produced by chimeric gene transfer. GPI-modified proteins are a class of native cell surface molecules that can be exogenously reincorporated back into cell membranes after purification (Medof et al., 160 J. Exp. Med. 1558, 1984; Moran et al., 149 J. Immunol. 1736, 1992; Zhang et al., 89 Proc. Natl. Acad. Sci. USA 5231, 1992; Bulow et al., 27 Biochemistry 2384, 1988; Hitsumoto et al., 5 Int. Immunol. 805, 1993). This property stems from their amphiphilic properties and their solubility in exceedingly low detergent concentration or in the complete absence of detergent. Protein transfer has been reported for a limited set of natural GPI-anchored proteins, including decay-accelerating factor ("DAF") (Medof et al., 160 J. Exp. Med. 1558, 1984; Moran et al., 149 J. Immunol. 1736, 1992), Thy-1 (Zhang et al., 89 Proc. Natl. Acad. Sci. USA 5231, 1992), *T. brucei* variant surface glycoprotein (Bulow et al., 27 Biochemistry 2384, 1988), and mouse heat-stable antigen (Hitsumoto et al., 5 Int. Immunol. 805, 1993). For DAF and heat stable antigen, biological functions have been demonstrated for the exogenously reincorporated proteins (Moran et al., 149 J. Immunol. 1736, 1992; Zhang et al., 89 Proc. Natl. Acad. Sci. USA 5231, 1992; Hitsumoto et al., 5 Int. Immunol. 805, 1993).

Polypeptide sequences can be artificially GPI-modified by linking their coding sequences to a GPI modification signal sequence (Tykocinski et al., 85 Proc.

Natl. Acad. Sci. USA 3555, 1988; Straus et al., 82 Proc. Natl. Acad. Sci. USA 6245, 1985; Caras et al., 238 Science 1280, 1987; Waneck et al., 85 Proc. Natl. Acad. Sci. USA 577, 1988). This finding has been substantiated by other more recent studies for a variety of target proteins (Lin et al., 249 Science 677, 1990; Sleckman et al., 147 J. Immunol. 428, 1991; Zhang et al., 115 J. Cell Biol. 75, 1991; Mann et al., 142 J. Immunol. 318, 1989; Matsui et al., 254 Science 1788, 1991; Wettstein et al., 174 J. Exp. Med. 219, 1991; Kemble et al., 122 J. Cell Biol. 1253, 1993; Weber et al., 210 Exp. Cell Res. 107, 1994; Huang et al., 31 Mol. Immunol. 1017, 1994; Scheirle et al., 149 J. Immunol. 1994, 1992). Gene transfer of GPI-modified MHC is disclosed by Mann et al., 142 J. Immunol. 318, 1989; Matsui et al., 254 Science 1788, 1991; Wettstein et al., 174 J. Exp. Med. 219, 1991; Scheirle et al., 149 J. Immunol. 1994, 1992; and Huang et al., 31 Mol. Immunol. 1017, 1994.

The coding sequence for the reagent of interest, for example, the extracellular domain of the Fas-L or single chain anti-class I Fv, can be linked in-frame to a GPI modification signal sequence from the carboxy terminus of a naturally GPI-modified protein such as human decay-accelerating factor. Such artificial GPI-modified proteins can be produced in large scale using mammalian (for example, glutamine synthetase amplification/expression system) or yeast (for example, Pichia expression system) over-expression systems. The artificial GPI-modified protein can then be purified by immunoaffinity chromatography or other standard biochemical purification methods. By incorporating a polyhistidine tag into the chimeric polypeptide, in between the two distinct coding sequences, one can simplify purification through the use of nickel-sepharose chromatography. Artificial GPI-modified inhibitors can be painted onto AVC surfaces by simply combining them with

the cells, optimally for one hour at room temperature or 37°C.

In the case of soluble reagent, protein transfer can be accomplished through the use of liposomes. Methods are well described for designing liposomes to be used as polypeptide carriers, and these can be directly applied to the delivery of immunoregulatory soluble molecule to AVCs. Alternatively, inert carriers, for example, polysaccharide beads, can be pre-coated with soluble molecules and delivered to the cytoplasm of cells. Additionally, APCs can be induced to pinocytose high concentrations of soluble molecules. In all of these cases, the immunoregulatory molecule is released by the APC slowly over time.

In another aspect, the invention features soluble inhibitory reagents that have been genetically engineered to confer special properties to them. One useful modification is to combine the sequences for more than one reagent into a single polypeptide. This provides for cooperative inhibitory functions without the need for using multiple reagents. In designing such "multi-functional reagents", one can draw upon published experience with chimeric hematopoietic cytokines which have been successfully used to modulate hematopoiesis. Another useful modification is lipid modifying reagents such as cytokines that normally exist as soluble forms in order to allow them to be anchored into the APC surface. Certain cytokines are known to function in both soluble and cell surface-associated modes. According to the present invention, immunosuppressive cytokines such IL-10 and TGF$\beta$ are produced in a lipid-modified, for example, GPI-modified, form and then coated onto APC surfaces to generate AVCs. Such tethered immunoregulatory cytokines retain the inhibitory function of their natural soluble counterparts.

In another aspect the invention features cells

other than conventional APCs that are engineered to function as AVCs. This involves cells that cannot themselves efficiently process complex polypeptide antigens. In such cells, antigen presentation is accomplished by either using endogenous MHC molecules which can be loaded (or pulsed) with exogenous antigenic peptides, or by transfecting the cells with MHC genes and then loading (or pulsing) the expressed proteins, or by coating the cells with pre-formed MHC:antigenic peptide complexes.

One example of a non-conventional APC that can be converted into an AVC is a grafted cell. Cells of a graft, such as epithelial cells, can be coated with an inhibitory molecule prior to transplantation into the recipient. This represents a method for promoting engraftment, since cytotoxic T-cell effectors can be ablated.

Other clinical applications for AVCs include a diverse set of autoimmune, alloimmune, and other acute and chronic inflammatory diseases. It is well-established in the scientific literature that pathogenic T-cells play critical roles in such diseases. AVCs can be administered to such patients, for example, intravenously, subcutaneously, intramuscularly, or intraperitoneally to inhibit such pathogenic T-cells. In the case of a patient suffering from an autoimmune disease, the patient's own APCs can be obtained, for example, by purifying them from phlebotomized blood via standard Ficoll-hypaque centrifugation and subsequent isolation methods. The cells are then converted into AVCs via inhibitory expression, pulsed with a source of pathogenic antigenic peptides, and then administered back to the patient. In the case of transplantation, the patient can be treated in a similar fashion with allogeneic AVCs prepared from the graft donor's blood. In preventing graft-versus-host disease, the transplanted marrow can be pre-treated with AVCs ex vivo.

In a first aspect the invention features a method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine release of a T-cell or inducing apoptosis or necrotic cell death in a T-cell directed to an alloantigen or processed antigen. The method entails providing an antigen presenting cell having a specific alloantigen or processed antigen. Then the extracellular surface of the antigen presenting cell is externally contacted with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind the chimera to the surface of the antigen presenting cell in a manner which presents the polypeptide on the cell's surface such that the polypeptide is able to reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

In an alternative embodiment the method entails inserting a genetic sequence encoding a non-CD8 cell surface polypeptide or a chimera into the antigen presenting cell.

Another embodiment of the method features inserting into an antigen presenting cell a genetic sequence encoding a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell.

In a further embodiment a secreted molecule is delivered to the antigen presenting cell in a form that allows for subsequent localized release.

By "artificial veto cell" is meant a non-naturally occurring cell that is capable of inhibiting the proliferation of, cytotoxicity of, or cytokine release by a T-cell or of inducing apoptosis or necrotic cell death in a T-cell. The cell bears an antigenic peptide at its

cell surface that can bind to the T-cell receptor of a T-cell that is being inhibited or induced to apoptose or undergo necrotic cell death. This antigenic peptide permits targeting of the inhibitory effect to antigen-specific T-cells. Generally, the cell is a molecularly modified conventional antigen-presenting cell, such as a dendritic cell or a B-cell. However, a non-antigen processing cell can also be converted to an artificial veto cell by providing an antigenic peptide to its cell surface.

By "proliferation" is meant cell division which increases the number of cells present. Cell division is associated with DNA synthesis and can be monitored ex vivo by measuring $^{3}H$-thymidine incorporation.

By "cytotoxicity" is meant the capacity of one cell to kill another cell. This cell function can be monitored ex vivo by measuring $^{51}Cr$ release from radio-labeled target cells.

By "cytokine secretion" is meant the release from the cell of a protein that mediates an immune response.

By "apoptosis" is meant programmed cell death in response to a variety of different triggers.

By "necrotic cell death" is meant cell death mediated by an environment made so hostile to the cell, by means of poisons or inappropriate pH or lack of oxygen, that the cell cannot maintain a state of homeostasis.

By "alloantigen" is meant either class I or class II MHC molecules with or without associated antigenic peptides from a different individual of the same species.

By "processed antigen" is meant fragments of a foreign substance, usually a protein, that bind to class I or class II MHC molecules.

An antigen presenting cell can be isolated in a number of ways. They may be obtained from peripheral blood. The blood is fractionated by density gradients to obtain either the mononuclear cells or the polynuclear

cells. This procedure is well known to persons skilled in the art. Various techniques can be utilized to isolate APCs (B lymphocytes, dendritic cells, or monocytes) such as adherence, adherence and release, fluorescence activated cell sorting with lineage specific antibodies, magnetic cell sorting with lineage specific antibodies, complement mediated killing with lineage specific antibodies. Polymorphonuclear blood cells can also be used as APCs. Various tissues also contain cells that can act as APCs. These tissues can be dissociated by physical and enzymatic means to release APCs. For example, endothelial cells can be obtained from vessels and myoblasts from muscle. The ability of some of these cells to act as APCs can be enhanced by treatment of the cells with cytokines such as interferon gamma. In the case of an alloantigen, isolation of the APC from a particular source will determine the type of alloantigen presented by the APC. A specific processed antigen can be expressed on an isolated APC by either feeding unprocessed antigenic peptide to the APC and allowing the APC to process the antigenic peptide or exposing the APC to processed antigen. Those in the art are familiar with both these techniques. In this manner, an antigen presenting cell having an alloantigen or processed antigen can be provided.

By "externally contacting the extracellular surface" is meant contacting the cell surface from the exterior as opposed to insertion into a cell membrane via an intracellular route.

By "chimera" is meant a polypeptide that possesses at least one domain from one protein and at least another domain from a different protein.

By "non-CD8 polypeptide" is meant a protein other than CD8, that is involved in immunoregulation by inhibition of cytokine release, proliferation or cytotoxicity or by inducing apoptosis or necrotic cell

death. Such molecules include: secreted molecules and cell surface associated molecules.

By "moiety sufficient to bind said chimera to said surface" is meant a molecule or domain of a molecule that allows for attachment to or incorporation into the cell membrane. For example, such a molecule may be a lipid.

By "inserting into said antigen presenting cell a genetic sequence" is meant any procedure that allows for introduction of nucleic acids into a cell, e.g., transfection, electroporation, liposome transfer.

By "genetic sequence encoding a non-CD8 cell surface polypeptide" is meant the sequence of nucleic acids specific for the non-CD8 cell surface polypeptide in the context of other regulatory sequences that enable the polypeptide sequence to be transcribed into mRNA and then translated into an active protein.

By "secreted molecule" is meant a molecule that can be released by an artificial veto cell so that it is free from the antigen presenting cell.

By "genetic sequence encoding a secreted molecule" is meant the sequence of nucleic acids specific for the secreted molecule in the context of other regulatory sequences that enable the molecules sequence to be transcribed into mRNA and then translated into an active protein.

By "locally released from said antigen presenting cell" is meant secreted in a soluble form in the extracellular space around the antigen presenting cell.

By "delivering a secreted molecule" is meant providing a secreted molecule in a form that allows for its uptake by an APC and subsequent local release.

In a preferred embodiment the non-CD8 polypeptide is selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC

heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, an anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor $\beta$, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein.

"Fas ligand" is a membrane bound polypeptide that is known to bind to a T-cell surface molecule Fas antigen and via this binding event induce apoptosis in said T-cell. (Brunner et al., 373 Nature 441, 1995; Dhein et al., 373 Nature 438, 1995; Ju et al., 373 Nature 444, 1995). The polypeptide to be used for AVC engineering comprises either the entire Fas ligand (amino acid residues 1-281) or the extracellular portion of Fas ligand (amino acid residues 103-281). (Takahashi et al. 6 Int. Immunol. 1567, 1994), incorporated herein by reference.

Fas antigen is defined as CD95, a cell surface molecule that binds Fas ligand.

By "anti-class I MHC heavy chain Ab" is meant an antibody molecule with specificity for the heavy polypeptide chain of a class I MHC heterodimer. Examples of anti-class I MHC antibodies that are capable of inhibiting a T-cell are 25.99, W6/32, CR1-S63, CR10-215, CR11-115, CR11-351, 5H7, Q6/64, Q1/28, 6/31, CR1, 01.65, and B1-23-2 (Tanabe, et al., 148 J. Immunol. 3202, 1992; De Felice, et al., 122 Cell. Immunol. 164, 1989; Smith, et al., 153 J. Immunol. 1054, 1994; Akiyama, et al., 91 Cell. Immunol. 477, 1985; Cavallini, et al., 154 Biochem. Biophys. Res. Commun. 723, 1988; Sterkers, et al., 131 J. Immunol. 2735, 1983). It is straightforward to clone the cDNAs corresponding to the immunoglobulin heavy and light chains from any one of the hybridomas expressing the respective anti-class I MHC heavy chain antibodies. These cDNAs can then be subcloned into expression vectors, and the resulting expression vectors can be used to express

the antibodies in cells being converted to artificial veto cells. For a membrane-associating variant, a membrane anchoring moiety can be appended to the carboxy-terminus. This can consist of a linker peptide, a hydrophobic transmembrane peptide and cytoplasmic extension (for example, an amino acid sequence corresponding to the hydrophobic transmembrane peptide and cytoplasmic extension of membrane IgG) or of a GPI modification signal sequence from a polypeptide that is naturally GPI-anchored to the membrane (for example, human decay-accelerating factor, spanning an amino acid sequence comprising Pro311 to Thr343 of this protein). Gene chimerization is readily carried out using splice-by-overlap-extension polymerase chain reaction ("SOE-PCR") technology. (Horton et al., 8 Biotechniques 528, 1990; Horton et al., 217 Methods Enzymol. 270, 1993).

By "anti-class I MHC heavy chain Fv chimeric polypeptide" is meant a recombinant fusion polypeptide comprising a leader peptide, the variable domain of the immunoglobulin heavy chain of an anti-class I MHC heavy chain Ab, a linker peptide (for example, $[(Gly)_3Ser]_3$), and the variable domain of the immunoglobulin light chain of an anti-class I MHC heavy chain Ab. For a membrane-associating variant, a membrane anchoring moiety is appended to the carboxy-terminus. This can consist of a linker peptide, a hydrophobic transmembrane peptide and cytoplasmic extension (for example, an amino acid sequence corresponding to the hydrophobic transmembrane peptide and cytoplasmic extension of membrane IgG) or of a GPI modification signal sequence from a polypeptide that is naturally GPI-anchored to the membrane (for example, human decay-accelerating factor, spanning an amino acid sequence comprising Pro311 to Thr348 of this protein). For a soluble Fv, a stop codon is appended immediately following the light chain variable domain.

By "anti-$\beta_2$ microglobulin antibody" is meant an

CLAIMS

1. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) externally contacting the extracellular surface of said antigen presenting cell with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind said chimera to said surface of said antigen presenting cell in a manner which presents said polypeptide on said cell's surface such that said polypeptide is able to reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

2. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) inserting into said antigen presenting cell a genetic sequence encoding a non-CD8 cell surface polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death, such that said polypeptide is expressed on said cell's surface so as to be able to

reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death.

3. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) inserting into said antigen presenting cell a genetic sequence encoding a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient so that said chimera is expressed on the surface of said antigen presenting cell in a manner which presents said polypeptide so as to be able to reduce T-cell proliferation or cytoxicity or induce T-cell apoptosis or necrotic cell death.

4. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) inserting into said antigen presenting cell a genetic sequence encoding a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell, such that said secreted molecule is locally released from said antigen presenting cell.

5. A method for producing an artificial veto cell capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death of a T-cell directed an alloantigen or processed antigen comprising the steps of:

a) providing an antigen presenting cell having said alloantigen or processed antigen, and

b) delivering a secreted molecule that is capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell, to the cytoplasm of said antigen presenting cell such that said secreted molecule is locally released from said antigen presenting cell.

6. A method for producing a non-naturally occurring biological membrane capable of specifically inhibiting the proliferation, cytotoxicity, cytokine secretion or inducing apoptosis or necrotic cell death in a T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) isolating a biological membrane having said alloantigen or processed antigen, and

b) contacting the extracellular portion of said biological membrane with a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient to bind said chimera to said surface of said biological membrane in a manner which presents said polypeptide on said biological membrane surface such that said polypeptide is able to reduce T-cell proliferation, cytoxicity, cytokine secretion or induce T-cell apoptosis or necrotic cell death.

7. A method for producing a non-naturally occurring biological membrane capable of specifically inhibiting the proliferation, cytotoxicity or cytokine secretion of a T-cell or inducing apoptosis or necrotic cell death in said T-cell directed to an alloantigen or processed antigen comprising the steps of:

a) isolating an antigen presenting cell having said alloantigen or processed antigen,

b) inserting into said antigen presenting cell a genetic sequence encoding a chimera comprising a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and a moiety sufficient so that said chimera is expressed on the surface of said antigen presenting cell in a manner which presents said polypeptide so as to be able to reduce T-cell proliferation, cytoxicity or cytokine secretion or induce T-cell apoptosis or necrotic cell death, and

c) isolating said biological membrane from said cell.

8. The method of claim 4, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor $\beta$, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

9. The method of claim 1, 2, 3, 6 or 7 wherein said polypeptide is selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor $\beta$, heterodimeric placental protein 14, homodimeric placental protein 14 , and a viral immunoregulatory protein.

10. The method of claim 5, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor $\beta$, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

11. The method of claim 1, 2, 3, 4, 5, 6 or 7 wherein an antigenic peptide presented by said antigen presenting cell is bound to a class I MHC polypeptide.

12. The method of claim 1, 2, 3, 4, 5, 6 or 7 wherein an antigenic peptide presented by said antigen presenting cell is bound to a class II MHC polypeptide.

13. The method of claim 1, 3, 6 or 7 wherein said moiety comprises a lipid modification.

14. The method of claim 13 wherein said lipid modification comprises glycosyl-phosphatidylinositol.

15. The method of claim 14 wherein said GPI-modified polypeptide is produced by the steps of:

a) gene transfer of a chimeric gene expression construct comprising a glycosyl-phosphatidylinositol modification signal sequence into a host cell; and

b) isolation of said GPI-modified polypeptide from said host cell.

16. The method of claim 5 wherein said secreted molecule is delivered to said antigen presenting cell via a liposome.

17. The method of claim 5 wherein said secreted molecule is delivered to said antigen presenting cell via an inert bead.

18. The method of claim 5 wherein said secreted molecule is delivered to said antigen presenting cell via pinocytosis.

19. A glycosyl-phosphatidylinositol-modified polypeptide comprising a polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from the extracellular domain of Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor $\beta$, heterodimeric placental protein 14, homodimeric placental protein 14 and an immunoregulatory viral protein, which is separate from a membrane.

20. An artificial veto cell having a membrane exogenously coated with a lipid-modified non CD8 polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from extracellular domain of Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor $\beta$, heterodimeric placental protein 14, homodimeric placental protein 14 and an immunoregulatory viral protein.

21. An artificial veto cell bearing a transfected genetic sequence that encodes a cell surface lipid modified non-CD8 polypeptide selected from the group consisting of polypeptides that comprise amino acid sequences from Fas ligand, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas antigen Fv chimeric polypeptide, interleukin-10, transforming growth factor $\beta$, heterodimeric placental protein 14, homodimeric placental protein 14, and an immunoregulatory viral protein.

22. An artificial veto cell bearing a transfected genetic sequence that encoding a secreted molecule capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion, or inducing apoptosis or necrotic cell death directed to alloantigens or processed antigens.

23. The method of claim 22, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor $\beta$, an anti-class I MHC heavy chain antibody, an anti-class I MHC

heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

24. An artificial veto cell containing an exogenously added secreted molecule capable of reducing T-cell proliferation, cytotoxicity, or cytokine secretion or inducing apoptosis or necrotic cell death directed to alloantigens or processed antigens.

25. The artificial veto cell of claim 24, wherein said secreted molecule is selected from the group consisting of interleukin-10, transforming growth factor $\beta$, an anti-class I MHC heavy chain antibody, an anti-class I MHC heavy chain Fv chimeric polypeptide, an anti-$\beta_2$ microglobulin antibody, and anti-$\beta_2$ microglobulin Fv chimeric polypeptide, an anti-Fas antigen antibody, an anti-Fas-antigen Fv chimeric polypeptide, a chimeric bacterial toxin molecule, HIV-tat, CTLA4Ig, a viral immunoregulatory protein, heterodimeric placental protein 14, homodimeric placental protein 14, and an antibody against a T-cell activation antigen.

26. A method for inhibiting an antigen-specific T-cell comprising the steps of:

a) providing an artificial veto cell which presents in, or on its surface a non-CD8 polypeptide capable of reducing T-cell proliferation, cytotoxicity or cytokine secretion or inducing T-cell apoptosis or necrotic cell death and an MHC:nominal antigen peptide complex, and